GUIDANCE[1]

DIFLUNISAL TABLETS

IN VIVO BIOEQUIVALENCE

AND *IN VITRO* DISSOLUTION

I. INTRODUCTION

A. Clinical Usage

Diflunisal is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory, and antipyretic properties. It is a salicylic acid derivative which produces longer-lasting responses than other comparative agents. Clinically, it is indicated for mild to moderate pain (initial dose of 1000 mg followed by 500 mg every 12 hours), osteoarthritis and rheumatoid arthritis (500-1000 mg daily in two divided doses) (1). For maintenance, a maximal daily dose of 1500 mg is recommended (1).

The precise mechanism of action of diflunisal is unknown, however, its therapeutic activity appears to be due to a decrease of prostaglandins in peripheral tissues. It also has a uricosuric effect. Analgesic doses of diflunisal increase renal clearance of uric acid and decrease serum uric acid (2,9).

Diflunisal is currently marketed as Dolobid [R] (Merck Sharp & Dohme) tablets in 250 mg and 500 mg strengths.

B. Chemistry

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[1]This statement, prepared by the Division of Bioequivalence in the Office of Generic Drugs, is an informal communication under 21 CFR 10.90(b)(9) that represents the best judgment of the Division at this time. This statement does not necessarily represent the formal position of the Center for Drug Evaluation and Research, Food and Drug Administration, and does not bind or otherwise obligate the Center for Drug Evaluation and Research, Food and Drug Administration, to the views expressed. For further information about this guidance, contact the Division of Bioequivalence, Office of Generic Drugs, 7500 Standish Place, Metro Park North, Rockville, MD 20855 (Phone: 301-295-8290; Fax: 301-295-8183).

Diflunisal is 2',4'-difluoro-4-hydroxy-3-biphenylcarboxylic acid. The chemical structure of diflunisal appears in the following figure:

It is a white crystalline material sparingly soluble in water and soluble in most organic solvents or dilute aqueous bases (1). The elimination of diflunisal is almost entirely dependent on glucuronidation of the parent compound although a sulfate conjugate has been identified in human volunteers after multiple dose administration (3).

C. Pharmacokinetics

Diflunisal is well absorbed after oral administration, peak plasma concentration being attained between 2 to 3 hours (4). The time required to each steady-state concentration increases with dosage from 3-4 days for a 125 mg bid dose to 7-9 days for a 500 mg bid dose (5). Available data indicate that diflunisal exhibits dose-dependent nonlinear pharmacokinetics over the dosing range of 250-1000 mg/day,(4-9) an effect that becomes more apparent after repetitive doses. Following single doses of 250 mg, 500 mg and 1000 mg, peak plasma concentration of 41, 87 and 124 μg/mL were observed respectively. However, following administration of 250 mg, bid, peak plasma concentration of 56 μg/mL at day 8 was observed; while 500 mg, bid, gave a peak plasma concentration of 190 μg/mL at day 11 (4).

More than 99% of diflunisal in plasma is bound to protein. The plasma half-life is reported to be 4 times longer than that of aspirin (8-12 hours) due to the difluorophenyl substitute on carbon 1 (4).

When diflunisal and indomethacin are given to normal volunteers, the renal clearance of indomethacin is decreased and plasma level is increased. Since fatal gastrointestinal hemorrhage has been associated with

the concomitant use of these drugs, they should not be administered concurrently (10). Concomitant administration of antacids and diflunisal may reduce the bioavailability of the latter drug; the effect is slight with occasional doses of antacids, but may be clinically significant during repeated administration (10). When taken with food, the absorption of diflunisal is delayed slightly but not decreased (10).

II. BIOEQUIVALENCE STUDIES

A. Types of Studies Required

1. A single-dose, fasting, two-way crossover study with the 500 mg tablet of generic diflunisal test product compared with the reference product, Dolobid® 500 mg tablet.

2. A single-dose, three-way crossover, limited food study with the 500 mg tablet of both generic diflunisal test product and reference product Dolobid® given to subjects under fed condition and the 500 mg tablet of the test product given to subjects under fasting condition.

3. *In Vitro* dissolution testing of the 500 mg strength tablets from test and reference lots used in the *in vivo* bioequivalence study.

4. *In Vitro* dissolution testing of the the 250 mg strength tablet.

B. Fasting Study

Objective: The objective of this study is to compare the bioavailability of a generic diflunisal 500 mg tablet (test product) with that of the reference product, Dolobid® 500 mg (Merck, Sharp & Dohme) under fasting conditions.

Design: The study design is a single dose, two treatment, two period, two sequence crossover with a washout period of at least 7 days. Subjects should be randomly assigned to the two possible dosing sequences.

Facilities: The clinical and analytical sites for the study should be given along with the names, titles and the curriculum vitae of the medical, scientific and

analytical directors. The starting and ending dates for each clinical study period should be stated. The study protocols should be approved by an institutional review board, and informed consent forms should be signed by all participants.

Subjects: The study should include 24 or more (to ensure adequate statistical results) adult, healthy, male volunteers from 19 to 45 years in age and within ±10% of the ideal weight for their height and body frame according to the Metropolitan Insurance Company Bulletin, 1983. All subjects should be given a physical examination and appropriate laboratory tests 4 weeks prior to the initiation of the study. These should be repeated at the end of the study.

Exclusion Criteria: Subjects should be excluded from the study using the following criteria and any other criteria deemed necessary by the medical director of the study:

1. History of hypersensitivity to diflunisal or related drugs;

2. History of serious hematological, cardiovascular, gastrointestinal, hepatic or renal diseases,

3. History of tuberculosis, asthma, psychosis or glaucoma,

4. History of alcoholism or drug abuse,

5. History of hypoalbuminemia,

6. Use of a prescription drug product within two weeks or any OTC drug product within 3 days of the start of the study,

7. Exposure to any agent known to induce or inhibit drug-metabolizing enzymes within 30 days prior to the study, and

8. Blood donation within 30 days prior to the study.

9. Tobacco use in any form.

Procedures: After an overnight (at least 10 hours) fast, subjects should receive a single dose of the test

product or the reference product with 240 ml of water:

Treatment A: Test product, 1 X 500 mg, diflunisal tablet

Treatment B: Reference product, 1 X 500 mg, Dolobid® (Merck, Sharp & Dohme) tablet

The test product should be from a production lot or from a lot produced under production conditions. The lot size of the test product should be equal to or more than 100,000. The lot numbers of both the test and reference products and the expiration date for the reference product should be stated. The potency of the reference product should not differ from that of the test product by more than ± 5%. The sponsor should include a statement of the composition of the test product.

The clinical staff administering the doses should verify that the dose was ingested by each subject. At least 7 days after the last sample collection in the first period of the study, each subject should receive the alternative treatment.

Restrictions: Prior to and during each study phase, subjects should conform to the following restrictions:

1. Water will be allowed *ad libitum* except for one hour before and after drug administration.

2. Subjects should be served standardized meals no less than 4 hours after drug administration. Only standardized meals and beverages at specified times will be allowed during the study.

3. No alcohol or xanthine-containing foods or beverages should be consumed for 48 hours prior to each study period and until after the last blood sample is collected.

4. Subjects will be confined to the clinical facility for 48 hours after each dosing.

Blood Sampling: Blood samples in volumes sufficient for sample analysis should be collected into appropriate anticoagulant containing tubes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 24, 36,

and 48 hours after dosing. Samples should be centrifuged promptly and plasma separated and frozen until assayed. The storage conditions and the time elapsed between sample collection and assay of each sample should stated. The stability of the drug in the plasma to be assayed during this period of time should be studied.

Analytical Methods: See Section V.

Pharmacokinetic Analysis: The plasma concentration of diflunisal of each subject at every sampling point should be reported for all subjects. The following pharmacokinetic parameters should also be obtained by the sponsor:

1. AUC_{0-t}, where T is the last measurable time point calculated by the trapezoidal rule.

2. $AUC_{0-\infty}$, where $AUC_{0-\infty} = AUC_t + C_t/(\lambda_Z)$, C_t is the last measurable drug concentration and λ_Z is the terminal elimination rate constant.

3. The terminal phase elimination rate constant (λ_z) is calculated using an appropriate pharmacokinetic method.

4. Peak drug concentration (C_{max}) and the time to peak drug concentration (T_{max}) are obtained directly from the data without interpolation.

Statistical Analysis: The sponsor should perform the following tests:

1. Analysis of variance (ANOVA) appropriate for a crossover design on the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} using General Linear Models (GLM) procedure of SAS(12) or an equivalent program should be performed. The statistical model should include terms describing the error attributable to sequence [subj (seq)], period and treatment. The sequence effect should be tested against the between subject [subj (seq)] error term. All other main effects should be tested against the residual error from the ANOVA.

2. The ESTIMATE statement in SAS should be used to obtain linear estimates for the adjusted

differences between treatment means and the error associated with these differences.

3. The LSMEANS statement should be used to calculate least-square means for treatments.

4. The two one-sided tests procedure (13) should be used to calculate 90% confidence intervals for the mean difference for AUC and C_{max}, which should generally be within $\pm$ 20% of the corresponding reference mean.

Adverse Reactions: The sponsor should report all adverse reactions that occurred during the study with regard to the nature, onset, duration, frequency, severity, type of treatment during which the reaction occurred and the suspected relation to the drug treatment.

C. Limited Food Effects Study

The labeling (4) for Dolobid ® recommends that diflunisal may be administered with water, milk or meals. Since diflunisal is an NSAID and physicians may also prescribe the drug to be taken with meals, a three-way crossover limited food study should be conducted using the same procedures as in the fasting study above, with the following exceptions:

a. The food study may be conducted in a minimum of 15-18 subjects with an equal number of subjects assigned to each of the six dosing sequences possible in the three-treatment study design.

b. Each subject will receive one of the following treatments:

Treatment A: test product, 1 X 500 mg, diflunisal tablet, given under fed conditions. [2]

Treatment B: reference product, 1 X 500 mg, Dolobid ® (Merck, Sharp & Dohme) tablet, given under fed conditions.

Treatment C: test product, 1 X 500 mg, diflunisal tablet, given under fasting conditions.

c. In general, the mean values of the parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for the test product should be within $\pm$ 20% of the corresponding mean values of the reference product.

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[2] Thirty minutes before drug administration, each subject should consume a standardized, high fat content meal consisting of:

one buttered English Muffin
one fried egg
one slice of American cheese
one slice of Canadian bacon
one serving of hash brown potatoes
Eight fluid oz. (240 mL) of whole milk
Six fluid oz. (180 mL) of orange juice

The meal should be completed before dosing.

III. IN VITRO DISSOLUTION TESTING REQUIREMENTS

A. Dissolution Testing

Dissolution testing should be conducted on 12 individual dosage units of the test and reference products from the same lots used in the *in vivo* bioequivalence studies using the following methodology:

Apparatus:	USP XXII Apparatus II (Paddle)
Speed:	50 rpm
Medium:	900 mL of 0.1M Tris buffer (Ph 7.2)
Temperature:	37°C
Sampling time:	10, 20, 30 and 45 minutes
Specification:	Not less than 80% of the labeled amount of the drug should be dissolved in 30 minutes.

The sponsor should include the following information from the dissolution testing:

1. Lot numbers for both test and reference products.

2. The percent dissolution for each dosage unit being tested at each time interval.

3. The mean percent dissolved, the range of percent dissolution and the coefficient of variation for the 12 units being tested at each time interval.

4. Validation data for the analytical method used.

5. Expiration date for the reference product.

B. Potency and Content Uniformity Determination

Prior to the initiation of the bioequivalence study, the applicant should determine the potency and content uniformity of the lot of the test and reference drug product to be used in the study. It is recommended that the applicant should ensure that the potency of the lot of the reference product is within 5% of that for the test product. The data on potency and content uniformity should be submitted with the dissolution data.

IV. WAIVER REQUEST

After approval of bioequivalence study results for the 500 mg tablet, a sponsor may submit a waiver request for the 250 mg strength of diflunisal tablet which should include the following:

a. A side-by-side comparison of the composition (names and quantities of active and inactive ingredients) of the 500 mg and the 250 mg tablet.

b. Dissolution data for the 250 mg tablets of the test product and the reference product.

V. ANALYTICAL METHODOLOGY

In recent studies, the two most commonly used methods for diflunisal analysis in plasma are both HPLC methods(7,11). In general, the sponsor should select a method with adequate specificity, accuracy, interday and intraday precision, linearity of standard curves and adequate sensitivity.

Quality control samples in the low, middle, and high ranges of the standard curve should be prepared (separate weighing for each control concentration) on the same day as the study samples are collected. Aliquots of the control samples should be stored frozen under the same conditions as the study samples. The lowest control should not exceed twice the concentration of the lowest standard. The lowest standard should be the sensitivity limit (limit of quantification).

In addition to evidence supporting the above, the sponsor should also submit the following:

a. Complete prestudy validation and detailed description of the analytical method.

b. Stability data from study conducted at:

    1. frozen conditions for at least as long as the longest period of time between sample collection and sample assay for the study,

    2. room temperature for at least as long as the longest period of time between sample thawing and sample assay, and

    3. freeze-thaw cycles if reassay is anticipated.

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